receptors as probes to determine formation and spatial distribution of individual FG-Nups in the native NPC with a spatiotemporal resolution of 9-10 nm and 400 μs. We found that Nup62 complex form a donut-like structure at the central scaffold of NPC and act as the primary selective gate for large single-independent cargoes. On either side of the central barrier, Nup98 and other FG-Nups could function as the secondary selective barrier for incoming molecules. Finally, the conformation of the central selective barriers can be significantly regulated by a major transport receptor Importin β1, but not by the other transport receptors including Importin β2, Crm1, NTF2 and CAS.

2678-Pos Board B448

Molecular Springs in the Nuclear Pore Complex of Live Cells

Francesco Cardarelli[1], Luca Lanzano[2], Enrico Gratton[2].
[1]Istituto Italiano di Tecnologia (IIT), Pisa, Italy, [2]Laboratory for Fluorescence Dynamics, Irvine, CA, USA.

Nuclear pore complexes (NPCs) are the gateways for nucleocytoplasmic exchange. Measurements of molecular transport through NPCs may provide valuable information to unravel the mechanism of communication between the nucleus and the cytoplasm. Unfortunately, because single molecules undergo very rapid transport, it is challenging to follow their motion in live cells. We set out to address the nanomechanical basis of pore function in intact cells by a combination of fluorescence correlation spectroscopy (FCS) and real-time tracking of the center of mass of single NPCs. We find the dynamics of the nucleoporin Nup153 to be regulated at the nanoscopic level so as to produce rapid, discrete exchange between two separate positions within the NPC. By means of the pair correlation function (pCF) analysis we are able to separate the two compnonents of Nup153 exchange: a fast collapse into compact molecular conformations (cytoplasm-to-nuclues) and a slightly slower release into extended conformations (nucleus-to-cytoplasm). We demonstrate that this signature activity is directly linked to the functional import of classical transport receptors and cargoes. Thus, we propose that the selective gating through intact NPCs is largely powered by spring-like molecular engines.

2679-Pos Board B449

Physical Modeling of the Conformational Dynamics of the Flexible Unfolded Proteins of the Nuclear Pore Complex

Anton Zilman[1], Michael Opferman[2], Rob Coalson[2], David Jasnow[2], Paul Welch[3].
[1]University of Toronto, Toronto, ON, Canada, [2]Pittsburgh University, Pittsburgh, PA, USA, [3]Los Alamos National Laboratory, Los Alamos, NM, USA.

Selective transport through the Nuclear Pore Complex (NPC) relies on the interactions of the transport factors with the natively unfolded proteins within the NPC channel (the FG nups). Despite recent advances, it is still not fully known how such transport can be mechanistically understood in terms of conformational dynamics of the FG nups controlled by the transport factors. Creation of artificial selective nano-channels functionalized with the FG nups (that mimic the NPC) or other flexible polymeric molecules extends the question of transport selectivity into a more general context.

Because many of the details of the conformational dynamics of the FG nups are not directly accessible experimentally on the relevant time scales, computational modeling is an important tool in addressing these questions. We present results of physical modeling of the effects of the transport factors on the conformational dynamics of FG nup-like flexible molecules in various geometries in order to explain experimental observations in vivo and in vitro. We establish which of the aspects of the conformational dynamics of the FG nups are essential for selective and efficient transport, and which are merely byproducts of the diffusion of the transport factors through a polymer-like medium. Finally, we propose a general coarse-grained description of selectivity in NPC-like channels.

2680-Pos Board B450

FRAP Analysis of Nuclear Export Rates Identifies Intrisic Features of Nucleocytoplasmic Transport

Francesco Cardarelli[1], Luca Tosti[2], Michela Serresi[1], Fabio Beltram[2,3], **Ranieri Bizzarri**[2,3].
[1]Center for Nanotechnology Innovation @ NEST, Istituto Italiano di Tecnologia, Pisa, Italy, [2]NEST, Scuola Normale Superiore, Pisa, Italy, [3]NEST, Istituto Nanoscienze - CNR, Pisa, Italy.

Aiming at a quantitative description of carrier-mediated nuclear export in live cells, we fused a prototypical leucine-rich nuclear export signal (NES) to GFP as a model cargo and expressed the fluorescent chimera into live CHO-K1 cells. The relevant parameters of NES-mediated nucleo-cytoplasmic transport were recovered by FRAP following an established theoretical description of kinetic exchanges between the cytoplasm and the nucleus. By our approach we were able to calculate the affinity of the expressed NES for the export machinery and the maximum rate of nuclear export achievable at saturation of endogenous carriers. Remarkably, the maximum export rate resulted similar to previously-determined maximum import rate; additionally, we demonstrated that export is not affected by the co-expression of saturating levels of a fluorescently-labeled nuclear import signal (NLS). Our results reveal the symmetry and dynamic decoupling between active export and import fluxes, thus highlighting the gating properties of single nuclear pores.

Voltage-gated Na Channels II

2681-Pos Board B451

A Proton Leak Current through the Cardiac Sodium Channel Linked to Mixed Arrhythmia and Dilated Cardiomyopathy Phenotypes

Pascal Gosselin-Badaroudine[1], Dagmar I. Keller[2], Hai Huang[1], Aurélien Chatelier[3], Mohamed Chahine[1].
[1]Laval University, Québec, QC, Canada, [2]University Hospital Basel, Basel, Switzerland, [3]Université de Poitiers, Poitiers, France.

Cardiac Na^+ channels encoded by *SCN5A* gene are essential for initiating heart beats and maintaining a regular heart rhythm. Mutations in these channels have recently been associated to atrial fibrillation, ventricular arrhythmias, conduction disorders and dilated cardiomyopathy (DCM).

We investigated a young male patient with a mixed phenotype composed of documented conduction disorder, atrial flutter, and ventricular tachycardia and DCM. Further family screening revealed DCM in the patient's mother his sister and in three of the mother's sisters. Because of the complex clinical phenotypes, we screened *SCN5A* and identified a novel mutation, R219H, which is located on a highly conserved region on the fourth helix of the voltage sensor domain of $Na_v1.5$. Three family members with DCM carried the R219H mutation.

The wild-type (WT) and mutant Na channels were expressed in a heterologous expression system and intracellular pH (pHi) was measured using a pH-sensitive electrode. The biophysical characterization of this mutant channel revealed an unexpected selective proton leak without any effect on the channel's biophysical properties. This H^+ leak through the mutated $Na_v1.5$ channel was not related to the Na^+ permeation pathway but occurred through an alternative pore. This pore most probably involves a proton wire on the voltage sensor domain.

We suggest that an acidification of cardiac myocytes and/or downstream events may cause the DCM phenotype and other electrical problems in affected family members. The identification of this clinically significant H^+ leak may lead to the development of more targeted treatment.

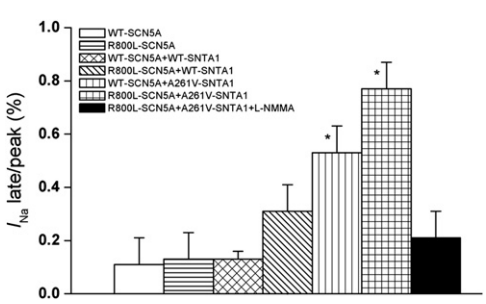
2682-Pos Board B452

Mutations in SNTA1 and SCN5A Interact to Increase Late Sodium Current in a Patient with Syncope

Roumu Hu[1], Bi-Hua Tan[1], Carmen Valdivia[1], Kate M. Orland[1], Amber Peterson[2], Yang He[1], Daniel J. Park[1], Jielin Pu[3], Jonathan C. Makielski[1].
[1]University of Wisconsin-Madison, Madison, WI, USA, [2]CentraCare Women and Children, St. Cloud, MN, USA, [3]Fu Wai Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China.

Alpha-1-Syntrophin (SNTA1) interacts with the C-terminus of the cardiac Na channel SCN5A and binds to neuronal nitric oxide synthase (nNOS) and the cardiac isoform of the plasma membrane Ca-ATPase (PMCA4b). We have reported that mutations in SNTA1 associated with a long QT syndrome patient and sudden infant death syndrome with increased late INa. Here, we characterize Na currents produced by A261V-SNTA1 and R800L-SCN5A found in a patient with syncope.

Comprehensive open reading frame/splice site mutational analysis of SCN5A and SNTA1 were performed using denaturing high performance liquid chromatography and DNA sequencing. We engineered R800L into the most common splice variant of SCN5A and A261V into SNTA1, transfected them in HEK293 cells along with nNOS and PMCA4b and measured late Na current by voltage clamp. The figure shows late INa as a percentage of peak INa increased by both mutations, the effects were additive, and blocked by the NG-monomethyl-L-arginie (L-NMMA), an NOS inhibitor.



This is only the second SNTA1 mutation in an adult associated with arrhythmia to increase late INa, and it interacts with an SCN5A mutation in an nNOS dependent mechanism.